

AMENDMENTS TO THE CLAIMS:

Claim 1. (Currently Amended) A process for the production of biologically active G-CSF, comprising:

expressing G-CSF as a heterologous protein in an expression system comprising a cultivated organism having at least one cell comprising at least one *E.Coli* bacterium cultivated at a temperature from about 20° C to about 30° C in the presence of an inducer, while regulating one or more of the following cultivation parameters: temperature of cultivation, composition of cultivation medium, induction mode, type of fermentation, addition of a stress induction agent, and co-expression of auxiliary proteins, wherein the protein is expressed as a substantially correctly folded G-CSF protein precursor in non-classical inclusion bodies, wherein the protein precursor has an aqueous solubility, and wherein regulating the cultivation parameters increases the proportion of substantially correctly folded G-CSF protein precursor present in the non-classical inclusion bodies in the cell, relative to the proportion of substantially correctly folded G-CSF protein precursor present in inclusion bodies in a cell of an organism not cultivated while regulating one or more of said parameters;

isolating the non-classical inclusion bodies containing the aforesaid correctly folded G-CSF protein precursor from the cell of the organism;

optionally, washing the non-classical inclusion bodies with a solution selected from the group consisting of Tris/HCl buffer, phosphate buffer, acetate buffer, citrate buffer, water, and combinations thereof,

solubilizing the substantially correctly folded G-CSF protein precursor isolated from the non-classical inclusion bodies under non-denaturing conditions by contacting the non-classical inclusion bodies aforesaid protein precursor with a liquid containing at least a non-denaturing aqueous solvent having a pH of about 8.0 below 10; and

purifying a the biologically active G-CSF protein from the solubilized substantially correctly folded G-CSF protein precursor and non-denaturing aqueous solvent,

wherein the process for the production of the biologically active G-CSF is free from any denaturation and renaturation of the G-CSF.

Claims 2 – 6. (Cancelled).

Claim 7. (Previously Presented) A process for the production of a protein according to claim 1, wherein the heterologous protein is accumulated in the inclusion bodies to a proportion of at least about 10%, relative to the total protein mass of a cell of the organism used in the expression system.

Claim 8 – 11. (Cancelled).

Claim 12. (Currently Amended) A process according to claim 1, wherein ~~regulating the induction mode comprises selecting an the inducer is selected~~ from the group consisting of IPTG, lactose, and NaCl, and combinations thereof.

Claim 13. (Previously Presented) A process according to claim 12, wherein the selected inducer is IPTG.

Claim 14. (Previously Presented) A process according to claim 13, wherein the concentration of IPTG ranges from about 0.1 mM to about 1 mM.

Claim 15. (Previously Presented) A process according to claim 14, wherein the concentration of IPTG is about 0.4 mM.

Claim 16. (Previously Presented) A process according to claim 12, wherein the regulation of the induction mode further comprises adding the inducer at the beginning of the fermentation.

Claim 17. (Previously Presented) A process according to claim 1, wherein the type of fermentation is selected from the group consisting of performing of fermentation in a batch mode, performing of fermentation in a fed batch mode, performing of fermentation in one or more shake flasks, and combinations thereof.

Claim 18. (Cancelled).

Claim 19. (Previously Presented) A process according to claim 1, wherein the composition of the cultivation medium is selected from the group consisting of GYST, GYSP, LYSP, LYST, LBON and GYSPO, and combinations thereof.

Claim 20. (Previously Presented) A process according to claim 19, wherein the selected medium is GYST or GYSP.

Claim 21. (Previously Presented) A process according to claim 1, wherein the stress induction agent is selected from the group consisting of ethanol, propanol, and combinations thereof.

Claims 22 – 23. (Cancelled).

Claim 24. (Currently Amended) A process according to claim 23 1, wherein the concentration of the selected buffer ranges from about 1 mM to about 10 mM.

Claim 25. (Previously Presented) A process according to claim 23, wherein the selected solution is water.

Claim 26. (Previously Presented) A process for production of a protein according to claim 1, wherein the non-denaturing aqueous solvent is selected from the group consisting of aqueous

solutions of: urea ranging in concentration from about 1M to about 2M, N-lauroyl sarcosine ranging in concentration from about 0.05% to about 0.25% mass per volume, betain, sarcosine, carbamoyl sarcosine, taurine, DMSO, non-detergent sulfobetains, a buffer in a high, solubilising concentration, and combinations thereof, said buffer being selected from the group consisting of HEPES, HEPPS, MES, and ACES, and combinations thereof.

Claim 27-37. (Cancelled).

Claim 38. (Previously Presented) The process of claim 26, wherein the non-denaturing aqueous solvent comprises a relatively low concentration of N-lauroyl sarcosine in water, in order to avoid denaturing conditions.

Claim 39. (Previously Presented) The process of claim 38, wherein the concentration of N-lauroyl sarcosine further ranges from about 0.1% to about 0.25% mass per volume.

Claim 40. (Previously Presented) The process of claim 1, wherein the specific activity of the G-CSF is at least 1×10^7 IU/mg.

Claim 41. (Previously Presented) The process of claim 1, wherein the amount of protein expressed is at least about 20% by mass of the total mass of proteins produced by the host cell.

Claim 42. (Previously Presented) The process of claim 1, wherein the amount of protein expressed is at least about 30% by mass of the total mass of proteins produced by the host cell.

Claim 43. (Currently Amended) A process for the production of biologically active G-CSF, comprising:

expressing ~~the~~ G-CSF as a heterologous protein in an expression system comprising at least one *E. coli* bacterium, while regulating ~~one or more of~~ the following cultivation parameters: ~~temperature of cultivation;~~ composition of cultivation medium, ~~induction mode;~~ type of fermentation, addition of a stress induction agent, and co-expression of auxiliary proteins, wherein the protein is expressed as a substantially correctly folded G-CSF protein precursor in non-classical inclusion bodies, wherein the protein precursor has an aqueous solubility, and wherein regulating the cultivation parameters increases the proportion of substantially correctly folded G-CSF protein precursor present in the non-classical inclusion bodies in the cell, relative to the proportion of substantially correctly folded G-CSF protein precursor present in inclusion bodies in a cell of a bacterium not cultivated while regulating ~~one or more of~~ said parameters;

isolating the non-classical inclusion bodies ~~containing the aforesaid correctly folded G-CSF protein precursor~~ from the cell of the organism;

optionally, washing the non-classical inclusion bodies with a solution selected from the group consisting of Tris/HCl buffer, phosphate buffer, acetate buffer, citrate buffer, water, and combinations thereof;

solubilizing the substantially correctly folded G-CSF protein precursor isolated from the non-classical inclusion bodies under non-denaturing conditions by contacting the non-classical inclusion bodies aforesaid protein precursor with a liquid containing at least a non-denaturing aqueous solvent having a pH of about 8.0 below 10; and

purifying the biologically active G-CSF protein from the solubilized substantially correctly folded protein precursor and non-denaturing aqueous solvent,

wherein the process for the production of the biologically active G-CSF is free from any denaturation and renaturation of the G-CSF

and wherein the temperature of cultivation is from about 20° C to about 30° C, the type of fermentation is fed-batch, and the induction mode is regulated using IPTG as an inducer.

Claim 44. (Previously Presented) A process for the production of biologically active G-CSF according to claim 43, wherein the G-CSF is accumulated in the inclusion bodies to a proportion of at least about 30%, relative to the total protein mass of a cell of the *E. coli* used in the expression system.

Claim 45. (Previously Presented) A process according to claim 43, wherein the concentration of IPTG ranges from about 0.1 mM to about 1 mM.

Claim 46. (Previously Presented) A process according to claim 45, wherein the concentration of IPTG is about 0.4 mM.

Claim 47. (Previously Presented) A process according to claim 43, wherein the composition of the cultivation medium is selected from the group consisting of GYST, GYSP, LYSP, LYST, LBON and GYSPO, and combinations thereof.

Claim 48. (Previously Presented) A process according to claim 43, wherein the stress induction agent is selected from the group consisting of ethanol, propanol, and combinations thereof.

Claim 49. (Cancelled).

Claim 50. (Currently Amended) A process according to claim 49 43, wherein the concentration of the selected buffer ranges from about 1 mM to about 10 mM.

Claim 51. (Currently Amended) A process according to claim 49 43, wherein the selected solution is water.

Claim 52. (Previously Presented) A process for production of a protein according to claim 43, wherein the non-denaturing aqueous solvent is selected from the group consisting of aqueous solutions of: urea ranging in concentration from about 1M to about 2M, N-lauroyl sarcosine ranging in concentration from about 0.05% to about 0.25% mass per volume, betain, sarcosine, carbamoyl sarcosine, taurine, DMSO, non-detergent sulfobetains, a buffer in a high, solubilising concentration, and combinations thereof, said buffer being selected from the group consisting of HEPES, HEPPS, MES, and ACES, and combinations thereof.

Claim 53. (Previously Presented) The process of claim 43, wherein the non-denaturing aqueous solvent comprises a relatively low concentration of N-lauroyl sarcosine in water, in order to avoid denaturing conditions.

Claim 54. (Previously Presented) The process of claim 53, wherein the concentration of N-lauroyl sarcosine further ranges from about 0.1% to about 0.25% mass per volume.

Claim 55. (Previously Presented) The process of claim 43, wherein the specific activity of the G-CSF is at least 1×10^7 IU/mg.